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Article Addendum

Akt signaling dynamics in plasma membrane microdomains visualized by FRET-based reporters

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Abbreviations: $PI(3,4,5)P_3$, phosphatidylinositol (3,4,5)-trisphosphate; PI3K, phosphatidylinositol 3-kinase; PDGF, platelet-derived growth factor; IGF-1, insulin-like growth factor-1; FRET, fluorescence resonance energy transfer; MβCD, methyl-β-cyclodextrin; AktAR, Akt activity reporter; InPAkt, indicator for 3' phosphoinositides based on Akt

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Human kinase Akt has been shown to be activated at the plasma membrane upon generation of the cell membrane bound second messenger phosphatidylinositol (3,4,5)-trisphosphate through phosphatidylinositol 3-kinase (PI3K). Several components in the PI3K/Akt signaling pathway have been found in the detergent-resistant plasma membrane compartments, lipid rafts. Increasing evidence also suggests crucial roles of lipid rafts in the activation of Akt in different cell types. However, the regulatory mechanisms of Akt activation at different microdomains of the plasma membrane are not clear. Using a newly developed genetically encodable fluorescent kinase reporter based on fluorescence resonance energy transfer (FRET), AktAR, we studied spatio-temporal dynamics of Akt activity within plasma membrane microdomains in live-cell context. Our studies suggest that Akt activity is turned on more rapidly in lipid rafts upon growth factor stimulation, and platelet-derived growth factor (PDGF) or insulin-like Growth Factor-1 (IGF-1) stimulated Akt activity is differentially regulated between raft and non-raft regions of the plasma membrane.

Since the identification of Akt as an oncogene in 1991,[1,2] much attention has been devoted to this kinase due to its pivotal roles in regulating cell growth, survival, motility, metabolism and proliferation.[3,4] Stimulation of cells with growth factors, cytokines, hormones or neurotransmitters leads to phosphatidylinositol 3-kinase (PI3K) generating the membrane associated second messenger phosphatidylinositol (3,4,5)-trisphosphate ($PI(3,4,5)P_3$), which promotes the translocation of Akt from the cytosol to the plasma membrane, where Akt is activated through phosphorylation of Thr308 in the activation loop and Ser473 within the hydrophobic motif.[5] The importance for Akt membrane binding during its activation was supported by previous investigations showing constitutive kinase activity upon membrane targeting of Akt with a myristoylated sequence motif.[6] Interestingly, myristoylated Akt was found to be highly enriched in cholesterol and sphingolipid-enriched detergent-resistant compartments, designated as lipid rafts.[7] Several components upstream of Akt signaling have also been reported as partitioned into the detergent-resistant membrane compartments.[8,9] Taken together, these observations suggest a possible involvement of rafts in PI3K/Akt pathway activation. However, the activation of Akt in different plasma membrane microdomains has not been studied in the cellular context, partially due to the lack of methods and tools suitable for dissecting signaling mechanisms in a complex and dynamic cellular environment. Using a newly developed genetically encodable fluorescent kinase reporter based on fluorescence resonance energy transfer (FRET), AktAR, we studied spatio-temporal dynamics of Akt activity within plasma membrane microdomains in live-cell context, showing platelet-derived growth factor (PDGF) or insulin-like Growth Factor-1 (IGF-1) stimulated Akt activity is differentially regulated between raft and non-raft regions of the plasma membrane.[10]

By using two subcellular targeted AktARs, PM(Lyn)-AktAR and AktAR-PM(Kras), we revealed the critical role of lipid rafts in Akt activation. Disruption of lipid rafts with methyl-β-cyclodextrin (MβCD) reduced the response of AktAR, demonstrating the integrity of lipid rafts is essential for full activation of Akt. The faster kinetics of the lipid raft associated PM(Lyn)-AktAR than the non-raft targeted AktAR-PM(Kras) indicate Akt activity is turned on more rapidly in lipid rafts upon growth factor stimulation.[10] Consistent with our finding, it has been shown that raft disruption selectively inhibited activation of Akt in response to stem cell factor stimulation in small cell lung cancer cells.[11] A recent study also showed plasma membrane microdomain impediment with inhibition of sphingolipid and cholesterol synthesis strongly reduced membrane recruitment of Akt in Jurkat cells.[12] Our investigation broadened current understanding of Akt signaling in plasma membrane microdomains by providing the first direct comparison of the activity dynamics of two pools of

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Akt in these compartments in living cells. Studies in small cell lung cancer cells and LNCaP cells demonstrated membrane Akt is mainly distributed in the non-raft regions, regardless of the low activity of non-raft associated Akt in these cells.[7,11] Thus, the high activity of raft Akt might be due to efficient interaction among components of the signaling pathway including the receptors, PI3K isoforms and Akt itself.

We then tested the roles of lipid rafts in different growth factor stimulated Akt signaling. Disruption of membrane rafts by MβCD diminished the PDGF stimulated response of PM(Lyn)-AktAR, without affecting that of AktAR-PM(Kras). Interestingly, cholesterol depletion with MβCD completely abolished IGF-1 stimulated response of AktAR-PM(Kras), indicating Akt signaling in the non-raft regions is dependent on that in the raft regions with IGF-1 stimulation.[10] By using the 3' phosphoinositide indicator, InPAkt (*in*dicator for 3' *p*hosphoinositides based on *Akt*),[13] we showed the integrity of lipid rafts is essential in maintaining cellular PI(3,4,5)P_3 production. To directly analyze the non-raft associated phosphoinositide production, we targeted InPAkt to the non-raft regions.[13] InPAkt-Kras gave a large response upon stimulation of PDGF, which was not affected by raft disruption. By sharp contrast, IGF-1 did not induce any detectable FRET changes of InPAkt-Kras. The data indicate the differential regulation pattern between PDGF and IGF-1 stimulated Akt signaling can be seen at the phosphoinositide level.[10] Therefore, we propose that rafts are the exclusive sites for IGF-1 stimulated 3' phosphoinositide production, while PDGF stimulates two different pools of 3' phosphoinositides, which initiates the activation of two relatively independent populations of Akt (Fig. 1). The different dynamics between these two growth factor mediated Akt signaling may result from differential coupling of the activated receptors to distinct PI3K isoforms. For instance, PDGF induced membrane ruffling is preferentially maintained by p110α, while insulin induced membrane ruffling is mainly maintained by p110β.[14]

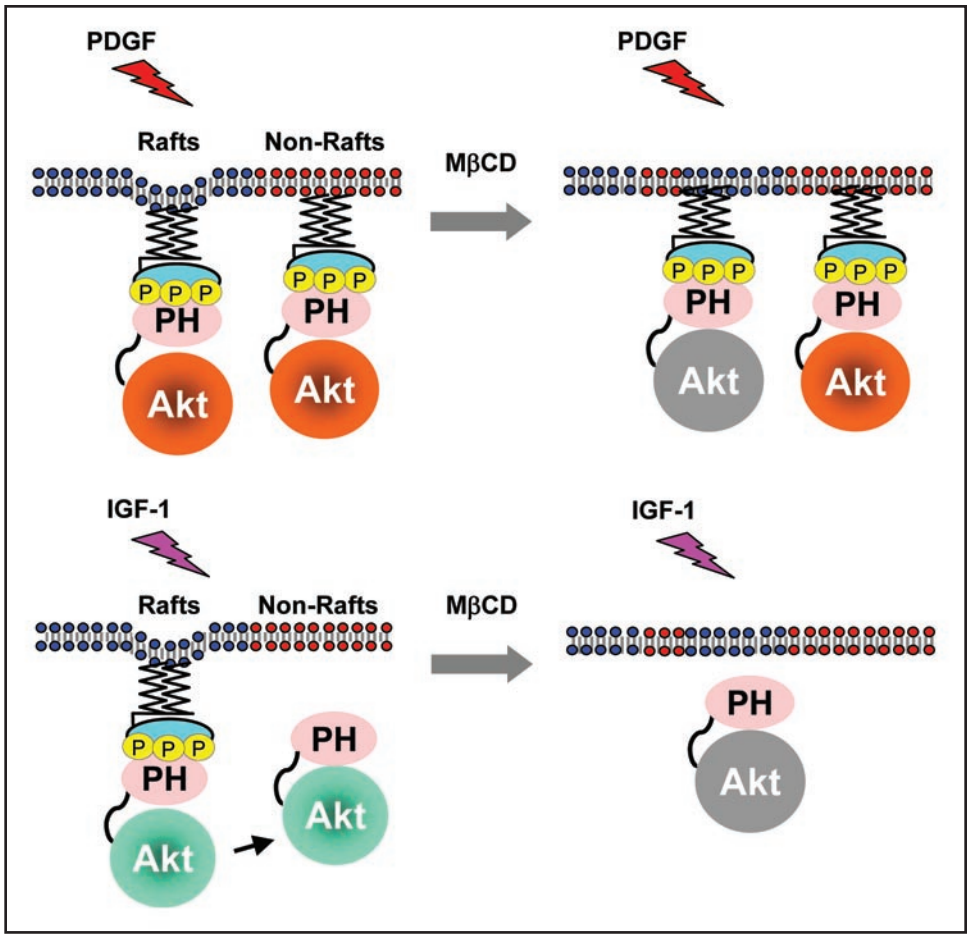

Figure 1. Models for regulation of Akt activity in different plasma membrane microdomains by PDGF and IGF-1. Lipid rafts are critical in regulating both PDGF and IGF-1 stimulated Akt activation. PDGF stimulates two pools of PI(3,4,5)P_3, regulating raft and non-raft Akt independently. Disruption of lipid rafts has minimum effect on non-raft Akt activity. IGF-1 stimulates PI(3,4,5)P_3 production mainly in lipid rafts. Therefore, Akt signaling in the non-raft regions is highly dependent on that in the raft regions.

It has been indicated that raft-associated Akt could be an important determinant of oncogenicity.[7] Previous observations showed localization and activity of myristoylated Akt1, which induces oncogenic transformation in chicken embryo fibroblast cultures,[6] can be regulated by manipulating membrane cholesterol levels,[7] suggesting a direct link between cholesterol levels and Akt oncogenic signaling pathway. Not coincidentally, epidemiologic investigations convey that the cholesterol-lowering statins could be potentially cancer preventive, therefore may complement cytotoxic chemotherapy/radiotherapy in established cancers.[15] Our data further imply such cholesterol-lowering drugs could display various drug efficacies in different tissues due to different cholesterol sensitivities of growth factor signaling pathways.[16,17]

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